

## WEST Search History

DATE: Wednesday, June 23, 2004

<b>Hide?</b>	<b>Set Name</b>	<b>Query</b>	<b>Hit Count</b>
<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND</i>			
<input type="checkbox"/>	L1	titball.in. and clostrid\$	11
<input type="checkbox"/>	L2	11 and secret\$	4
<input type="checkbox"/>	L3	11 and signal\$	3
<input type="checkbox"/>	L4	L3 or 12	6
<input type="checkbox"/>	L5	14 and (secreti\$ or signal\$)	4

END OF SEARCH HISTORY

The etx gene encoding epsilon toxin is carried out on an episome distinct from the 3.6Mb chromosome (Canard, B., Saint Joanis, B., and Cole, S. T. (1992); Genomic diversity and organization of virulence genes in the pathogenic anaerobe *Clostridium perfringens*. Mol. Microbiol. 6, 1421-1429). The gene has been cloned and sequences for both B and D types determined. The cloned gene etxB coded for a protein of M.sub.r.about.32,981 (Hunter, S. E., Clarke, I. N., Kelly, D. C., and Titball, R. W. (1992); Cloning and nucleotide sequencing of the *Clostridium perfringens* epsilon-toxin gene and its expression in *Escherichia coli*; Infect. Immun. 60, 102-110). Neither the sequenced gene or the derived protein showed homology with other proteins. Comparison of the sequences of cloned etx genes from type B and type D strains revealed two nucleotide differences in the open reading frame resulting in one amino acid substitution (Havard, H. L., Hunter, S. E., and Titball, R. W. (1992); Comparison of the nucleotide sequence and development of a PCR test for the epsilon toxin gene of *Clostridium perfringens* type B and type D; FEMS Microbiol. Lett. 76, 77-81). The promoters for the genes were not homologous, with different putative -10 and -35 sequences. This allowed the development of epsilon-specific PCR primers to produce a system for typing B and D strains of *C. perfringens*. The etx promoter allowed expression of the cloned gene in *E. coli* (Hunter et al., 1992). Epsilon toxin is preceded by a signal peptide resulting in the native protein being exported from *C. perfringens* and the recombinant protein accumulating in the periplasmic space of *E. coli* (Hunter et al., 1992; Bullen, J. J. and Batty, I. (1956); The effect of *Clostridium welchii* type D culture filtrates on the permeability of the mouse intestine; J. Pathol. Bacteriol. 71, 311-323). The recombinant toxin expressed in *E. coli* was shown to have identical biochemical and biological properties to those of the native toxin.

## WEST Search History

DATE: Wednesday, June 23, 2004

<u>Hide?</u>	<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>
		<i>DB=USPT; PLUR=YES; OP=AND</i>	
<input type="checkbox"/>	L1	secreti\$ near3 signal\$ near2 \$peptide	1173
<input type="checkbox"/>	L2	L1 and clostrid\$	99
<input type="checkbox"/>	L3	L1 same clostrid\$	1
<input type="checkbox"/>	L4	L1 same clostrid\$	1
<input type="checkbox"/>	L5	L1.clm. and clostrid\$.clm. not l4	2
<input type="checkbox"/>	L6	signal\$ near2 \$peptide	8632
<input type="checkbox"/>	L7	L6 same clostrid\$	13
<input type="checkbox"/>	L8	L7 not l3 not l4	12
<input type="checkbox"/>	L9	secreti\$.ti,ab,clm. near3 \$peptide.ti,ab,clm.	239
<input type="checkbox"/>	L10	L9 and clostrid\$	16
<input type="checkbox"/>	L11	L10 not l7 not l8 not l3 not l4	15

END OF SEARCH HISTORY

## WEST Search History

DATE: Wednesday, June 23, 2004

<u>Hide?</u>	<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>
		<i>DB=USPT; PLUR=YES; OP=AND</i>	
<input type="checkbox"/>	L1	5874220.pn. and promoter	0
<input type="checkbox"/>	L2	5874220.pn. and transcrip\$	1
<input type="checkbox"/>	L3	5874220.pn. and codon and start	0
<input type="checkbox"/>	L4	5874220.pn. and codon and ribosom\$	0
<input type="checkbox"/>	L5	5874220.pn. and codon	1

END OF SEARCH HISTORY

[Generate Collection](#)[Print](#)**Search Results** - Record(s) 1 through 13 of 13 returned.

- 
- ☐ 1. [6713617](#). 30 Nov 00; 30 Mar 04. Cloning, sequencing and expression of a gene encoding an eukaryotic amino acid racemase, and diagnostic, therapeutic, and vaccination applications of parasite and viral mitogens. Minoprio; Paola, et al. 536/23.2; 435/183 536/23.1 536/23.7. C07H021/04 C07H021/02 C12N009/00.
- 
- ☐ 2. [6652849](#). 17 May 02; 25 Nov 03. Anaerobe targeted enzyme mediated prodrug therapy. Brown; John Martin, et al. 424/93.2; 424/93.4 424/93.41 435/243 435/252.1 435/252.7 435/320.1. A01N063/00 A61K048/00.
- 
- ☐ 3. [6416754](#). 23 Jul 96; 09 Jul 02. Anaerobe targeted enzyme-mediated prodrug therapy. Brown; John Martin, et al. 424/93.21; 435/191 435/206 435/227 435/252.3 435/252.7 435/320.1 435/325 435/357 435/366 435/367 514/44 536/23.2. A61K048/00.
- 
- ☐ 4. [6403094](#). 11 Sep 98; 11 Jun 02. Clostridium perfringens vaccines. Titball; Richard W, et al. 424/190.1; 424/192.1 424/197.11 424/234.1 424/236.1 424/239.1 424/247.1 530/350 530/403 530/820 530/825. A61K039/02 A61K039/00 A61K039/385 A61K039/08 C07K001/00.
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- ☐ 5. [6350591](#). 16 Feb 99; 26 Feb 02. Recombinant DNA and methods for producing thermostable enzymes. Weber; J. Mark, et al. 435/69.1; 435/477 536/23.7. C12N015/74 C12N015/31.
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- ☐ 6. [6280993](#). 24 Aug 99; 28 Aug 01. Gene encoding class I collagenase. Yamato; Ichiro, et al. 435/220; 435/252.3 435/252.33 435/320.1 435/69.1 435/69.7 536/23.2 536/23.4. C12N009/52 C12N015/57 C12N015/70.
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- ☐ 7. [6271011](#). 21 Sep 99; 07 Aug 01. Pasteurella neuraminidase coding sequences and diagnostic methods. Lee; Margie, et al. 435/200; 435/252.3 435/320.1 435/325 435/69.1 536/23.2 536/24.3. C12N009/24 C12N001/20 C12N015/00 C12P021/06 C07H021/04.
- 
- ☐ 8. [6150139](#). 25 Feb 99; 21 Nov 00. Bacterocide compositions and their preparation from micrococcus varians. Mollet; Beat, et al. 435/71.2; 435/252.1 530/324. C12P021/02 C12N001/22 C07K014/305.
- 
- ☐ 9. [6096546](#). 30 Jan 98; 01 Aug 00. Methods for recovering polypeptides from plants and portions thereof. Raskin; Ilya. 435/410; 210/600 435/68.1 435/69.1 435/70.1. C12N005/00.
- 
- ☐ 10. [5955258](#). 22 Apr 97; 21 Sep 99. Process for the lysis of a culture of lactic acid bacteria by means of a lysin, and uses of the resulting lysed culture. Buist; Girbe, et al. 435/4; 424/93.21 424/94.61 435/252.1 435/320.1 435/6 435/69.1 435/71.2 435/91.1 536/23.2 536/23.7 536/24.1. C12Q001/00 C12N001/00 A01N063/00 C07H021/04.
- 
- ☐ 11. [5872238](#). 18 Aug 97; 16 Feb 99. Thermophile gene transfer. Weber; J. Mark, et al. 536/23.7;. C12N015/31.
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- ☐ 12. [5786174](#). 28 Jan 97; 28 Jul 98. Thermophile gene transfer. Weber; J. Mark, et al. 435/69.1; 435/463 530/350 536/23.1. C12P021/02 C12N015/63 C07K014/00 C07H021/04.
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☐ 13. 5004692. 15 Dec 87; 02 Apr 91. Cloning and expression of phospholipase C genes. Tso; J. Yun, et al. 435/183; 435/195 435/252.3 435/252.33 435/320.1 435/358 435/365 435/367 536/23.2 536/23.7. C12N009/00 C12N009/14.

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Terms	Documents
L6 same clostrid\$	13

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First Hit   Fwd Refs

L7: Entry 1 of 13

File: USPT

Mar 30, 2004

US-PAT-NO: 6713617

DOCUMENT-IDENTIFIER: US 6713617 B2

TITLE: Cloning, sequencing and expression of a gene encoding an eukaryotic amino acid racemase, and diagnostic, therapeutic, and vaccination applications of parasite parasite and viral mitogens

DATE-ISSUED: March 30, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Minoprio; Paola	Villiers s/Marne			FR
Arala-Chaves; Mario	late of Lisboa			PT
Coutinho; Antonio	Paris			FR
San Martin; Bernardo Reina	Gentilly			FR
Rougeot; Catherine	Chevreuse			FR
DeGrave; Wim	Bagneu			FR
Cosson; Alain	Neuilly S/Marne			FR

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Institut Pasteur	Paris			FR	03

APPL-NO: 09/ 725945   [PALM]

DATE FILED: November 30, 2000

## PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is based on and claims the benefit of each of the following applications: U.S. Provisional Application Ser. No. 60/168,631, filed Dec. 3, 1999; U.S. Provisional Application Ser. No. 60/220,207, filed Jul. 24, 2000; and U.S. Provisional Application S.No. 60/221,117, filed Jul. 27, 2000. The entire disclosure of each of these applications is relied upon and incorporated by reference herein.

INT-CL: [07] C07 H 21/04, C07 H 21/02, C12 N 9/00US-CL-ISSUED: 536/23.2; 536/23.1, 536/23.7, 435/183US-CL-CURRENT: 536/23.2; 435/183, 536/23.1, 536/23.7FIELD-OF-SEARCH: 424/269.1, 435/7.22, 435/6, 435/183, 536/23.1, 536/23.2, 536/23.5, 536/23.7, 536/24.1, 536/24.2, 536/24.3, 536/24.32, 536/24.33

## PRIOR-ART-DISCLOSED:

## OTHER PUBLICATIONS

Sequence alignments and EMBL-EBI sequence listing for Accession Nos., AA952585,

AA952632, AA882923, AI057768, AI057684, AI562488, AI053153, AI057805, AQ908238 and AQ903011.\*

Verden et al, Infection and Immunity, Nov. 1998, p. 5393-5398.

ART-UNIT: 1645

PRIMARY-EXAMINER: Duffy; Patricia A.

ASSISTANT-EXAMINER: Ford; Vanessa L.

ATTY-AGENT-FIRM: Finnegan Henderson Farabow Garrett & Dunner, LLP

ABSTRACT:

A method of preventing or inhibiting infection by a parasite or virus in vivo comprises administering to a human in need thereof a parasite or virus mitogen in a sub-mitogenic amount sufficient to induce a protective immune response against the parasite or virus in the human.

5 Claims, 43 Drawing figures



## Hit List

Clear	Generate Collection	Print	Fwd Refs	Bkwd Refs
Generate OACS				

Search Results - Record(s) 1 through 12 of 12 returned.

☐ 1. Document ID: US 6652849 B2

L8: Entry 1 of 12

File: USPT

Nov 25, 2003

DOCUMENT-IDENTIFIER: US 6652849 B2

**\*\* See image for Certificate of Correction \*\***

TITLE: Anaerobe targeted enzyme mediated prodrug therapy

Detailed Description Text (61):

Unlike NTR, where access to bacterial cytoplasmic co-factors is adventitious, for activity the .beta.-glucuronidase has to be exported out of the clostridial cell. To achieve this, the uidA gene is endowed with a 5' signal sequence to promote secretion of the translated protein by the classical signal peptide route (Bosslet et al. (1992) Br. J. Cancer 65:234-238). Two signal sequences are employed: (1) the Clostridium thermocellum celA gene (Beguín et al. (1985) J. Bacteriol. 162:102-105); and (2) the staphylococcal protein A gene (Shuttleworth et al. (1987) Gene 58:283-295). The 5'-end of the lacZ' gene of pMTL500F (that residing between the NdeI site and polylinker region, see FIG. 4) is replaced with DNA specifying the two signal sequences, approximately 10 or so codons past the signal peptidase encoded cleavage site. Thereafter, a suitably modified copy of the uidA gene (one in which a restriction site is introduced by site-directed mutagenesis at the second codon) is introduced into the polylinker region of the two resultant plasmids, such that in phase fusion occurs between the celA- or spa-derived sequences. The efficiency with which the resultant CelA::UidA and Spa::UidA fusion proteins are exported from C. acetobutylicum cells is assessed. Maximum efficiency of secretion and enzymic activity of the processed fusion protein, may require some experimentation in the number of celA/spa-derived amino acids at the NH2-terminus of the created fusion proteins.

Full	Title	Citation	Front	Review	Classification	Date	Reference	SEQUENCES	ATTACHMENTS	Claims	KWIC	Draw D
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☐ 2. Document ID: US 6416754 B1

L8: Entry 2 of 12

File: USPT

Jul 9, 2002

DOCUMENT-IDENTIFIER: US 6416754 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Anaerobe targeted enzyme-mediated prodrug therapy

Detailed Description Text (62):

Unlike NTR, where access to bacterial cytoplasmic co-factors is adventitious, for activity the .beta.-glucuronidase has to be exported out of the clostridial cell. To achieve this, the uidA gene is endowed with a 5' signal sequence to promote secretion of the translated protein by the classical signal peptide route (Bosslet et al. (1992) Br. J. Cancer 65:234-238). Two signal sequences are employed: (1) the Clostridium thermocellum celA gene (Beguin et al. (1985) J. Bacteriol. 162:102-105); 105); and (2) the staphylococcal protein A gene (Shuttleworth et al. (1987) Gene 58:283-295). The 5'-end of the lacZ' gene of pMTL500F (that residing between the NdeI site and polylinker region, see FIG. 4) is replaced with DNA specifying the two signal sequences, approximately 10 or so codons past the signal peptidase encoded cleavage site. Thereafter, a suitably modified copy of the uidA gene (one in which a restriction site is introduced by site-directed mutagenesis at the second codon) is introduced into the polylinker region of the two resultant plasmids, such that in phase fusion occurs between the celA- or spa-derived sequences. The efficiency with which the resultant Cella::Uida and Spa::Uida fusion proteins are exported from *C. acetobutylicum* cells is assessed. Maximum efficiency of secretion and enzymic activity of the processed fusion protein, may require some experimentation in the number of celA/spa-derived amino acids at the NH2-terminus of the created fusion proteins.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Abstracts	Claims	KMOC	Drawings
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☐ 3. Document ID: US 6403094 B1

L8: Entry 3 of 12

File: USPT

Jun 11, 2002

DOCUMENT-IDENTIFIER: US 6403094 B1

TITLE: *Clostridium perfringens* vaccines

Brief Summary Text (7):

The etx gene encoding epsilon toxin is carried out on an episome distinct from the 3.6Mb chromosome (Canard, B., Saint Joannis, B., and Cole, S. T. (1992); Genomic diversity and organization of virulence genes in the pathogenic anaerobe *Clostridium perfringens*. Mol. Microbiol. 6, 1421-1429). The gene has been cloned and sequences for both B and D types determined. The cloned gene etxB coded for a protein of M.sub.r.about.32,981 (Hunter, S. E., Clarke, I. N., Kelly, D. C., and Titball, R. W. (1992); Cloning and nucleotide sequencing of the *Clostridium perfringens* epsilon-toxin gene and its expression in *Escherichia coli*; Infect. Immun. 60, 102-110). Neither the sequenced gene or the derived protein showed homology with other proteins. Comparison of the sequences of cloned etx genes from type B and type D strains revealed two nucleotide differences in the open reading frame resulting in one amino acid substitution (Havard, H. L., Hunter, S. E., and Titball, R. W. (1992); Comparison of the nucleotide sequence and development of a PCR test for the epsilon toxin gene of *Clostridium perfringens* type B and type D; FEMS Microbiol. Lett. 76, 77-81). The promoters for the genes were not homologous, with different putative -10 and -35 sequences. This allowed the development of epsilon-specific PCR primers to produce a system for typing B and D strains of *C. perfringens*. The etx promoter allowed expression of the cloned gene in *E. coli* (Hunter et al., 1992). Epsilon toxin is preceded by a signal peptide resulting in the native protein being exported from *C. perfringens* and the recombinant protein accumulating in the periplasmic space of *E. coli* (Hunter et al., 1992; Bullen, J. J. and Batty, I. (1956); The effect of *Clostridium welchii* type D culture filtrates on the permeability of the mouse intestine; J. Pathol. Bacteriol. 71, 311-323). The recombinant toxin expressed in *E. coli* was shown to have identical biochemical and biological properties to those of the native toxin.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 4. Document ID: US 6350591 B1

L8: Entry 4 of 12

File: USPT

Feb 26, 2002

DOCUMENT-IDENTIFIER: US 6350591 B1

TITLE: Recombinant DNA and methods for producing thermostable enzymes

Brief Summary Text (19):

Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum *celA* Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431, teach the self-selection of undescribed origins of replication from cryptic plasmids from uncharacterized Thermus spp. and Thermus aquaticus are isolated and cloned into E. coli vectors. Plasmids were constructed with these origins, pLU1 to pLU4 from T. aquaticus, and pMY1 to pMY3 from Thermus spp. The plasmids then had a modified form of the cellulase gene (*celA*) from Clostridium thermocellum and were expressed in E. coli with the signal peptide from the S-layer gene from T. thermophilus. Transformation back into T. thermophilus allowed for expression at 70.degree. C.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 5. Document ID: US 6280993 B1

L8: Entry 5 of 12

File: USPT

Aug 28, 2001

DOCUMENT-IDENTIFIER: US 6280993 B1

TITLE: Gene encoding class I collagenase

Detailed Description Text (12):

As used herein, the term class I collagenase from Clostridium histolyticum means a polypeptide having 1118 amino acids of SEQ ID NO: 1. It is considered that the class I collagenase of SEQ ID NO: 1 is a premature protein, and contains a signal peptide consisting of the first to 109th amino acids of SEQ ID NO: 1, whereas a matured class I collagenase from Clostridium histolyticum consists of the 110th to 1118th amino acids of SEQ ID NO: 1.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 6. Document ID: US 6271011 B1

L8: Entry 6 of 12

File: USPT

Aug 7, 2001

DOCUMENT-IDENTIFIER: US 6271011 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Pasteurella neuraminidase coding sequences and diagnostic methods

Detailed Description Text (6):

While the *P. multocida* Nanli amino acid sequence has some similarity to the amino acid sequences of other bacterial neuraminidases, the exemplified DNA sequence does not exhibit significant homology to any other gene sequence deposited in GenBank. The open reading frame encodes a protein of approximately 47.4 to 50 kDa, including a signal peptide. It is possible that the discrepancy among published reports results from the isolation of aggregates or degradation products of the protein which may retain some enzymatic activity in those previous reports. Without wishing to be bound by theory, it is also possible that *P. multocida* produces more than one enzyme with neuraminidase activity, as has been seen in some clostridial species [Roggentin et al. (1993) Mol. Microbiol. 9(5):915-921].

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw D
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☐ 7. Document ID: US 6150139 A

L8: Entry 7 of 12

File: USPT

Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150139 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Bacterocide compositions and their preparation from micrococcus varians

Brief Summary Text (120):

Variants of the protein having the amino acid sequence SEQ ID NO:1 are created. To this end, the encoding DNA sequence of the variacin without the peptide signal (recombinant vector pK19 above) is inserted into the polyclonal site of plasmid pKK232-8 (Pharmacia, UK). Chemical mutagenesis with hydroxylamine are performed on the expression vector according to the process described by Yeast et al. (Applied and Env. Micro., 60, 1221-1226, 1994). Other methods could also be used, such as the method described by Dunn et al. (Protein Engineering, 2, 283-291, 1988) dealing with the creation of precise mutations. The mutagenized vectors are transferred into competent *E. coli* BZ 234 and transformants are selected. Transformants are isolated and cultivated. Supernatants are prepared and concentrated according to Example 2. Each supernatant is then screened according to the "agar well test" described above. Results show that some supernatants provide an inhibition activity against *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Listeria*, *Bacillus*, Clostridia and/or *Staphylococcus*, for example. DNA analysis of vectors expressing the bacteriocin show that some bacteriocins present a DNA sequence which is different to the encoding sequence SEQ ID NO:2. The amino acid sequence of these variants are generally different by from 1 to 4 amino acids.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw D
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☐ 8. Document ID: US 6096546 A

L8: Entry 8 of 12

File: USPT

Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096546 A

TITLE: Methods for recovering polypeptides from plants and portions thereof

Detailed Description Text (7):

Plasmid constructs designed for use in methods according to the invention are schematically illustrated in FIG. 1, wherein "Plasmid" indicates plasmid identities; "Promoter" identifies particular promoters (i.e., mas2' is the mannopine synthase promoter; 2.times.En35S is the Cauliflower Mosaic Virus 35S promoter with two enhancers; 35S is the Cauliflower Mosaic Virus 35S promoter; and Ubiquitin is the Maize Ubiquitin promoter); "Signal peptide" identifies the signal peptides encoded by the plasmids; and Restriction map provides schematic physical maps of the plasmids indicating coding regions and expression elements (i.e., TL- the TEV 5' nontranslated sequence; SP is a coding region for the relevant signal peptide; GFP is Green Fluorescent Protein, SEAP is Human Placental Secreted Alkaline Phosphatase, and Xylanase is the enzyme from *Clostridium thermocellum*). Restriction sites are indicated as follows: B-BamHI, Bg-Bg/II, Bs-Bst/BI, E-EcoRI, H-HindIII, K-KpnI, -NotI, Nc-NcoI, Nh-NheI, P-PstI, S-SacI, Sa-SalI, Sp-SphI.

Full	Title	Citation	Front	Review	Classification	Date	Reference	References	Attachment	Claims	KWIC	Draw. De
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☐ 9. Document ID: US 5955258 A

L8: Entry 9 of 12

File: USPT

Sep 21, 1999

DOCUMENT-IDENTIFIER: US 5955258 A

TITLE: Process for the lysis of a culture of lactic acid bacteria by means of a lysin, and uses of the resulting lysed culture

Detailed Description Text (71):

In this report we present the cloning of the first peptidoglycan hydrolase of the genome of a lactic acid bacterium. The gene encodes the major peptidoglycan hydrolase of *Lactococcus lactis*. Using PCR and denaturing SDS-PAGE the gene was detected in all *L. lactis* strains used and, in fact, in all strains tested so far (unpublished observation). Because the gene was also detected in strain AM1, in which Mou et al. (31) observed only a muramidase activity in the cell wall fraction, we conclude that the cloned gene encodes the lactococcal N-acetylmuramidase, an enzyme hydrolysing the linkages between N-acetylmuramic acid and N-acetylglucosamine moieties and was. Accordingly, the gene was designated acmA. Using a standardized assay to detect autolytic activity in a denaturing polyacrylamide gel, several lytic bands were found in both the cell and supernatant fractions of an *L. lactis* culture using *M. lysodeikticus* cell walls as a substrate, but only a few of these bands were found when cell walls of the host were used. This result indicates that the lytic activities of *L. lactis* are far better detectable with *M. lysodeikticus* cell walls as a substrate. This has also been observed by Leclerc et al. (26) when they analyzed bacterial extracts of *Clostridium perfringens*, *Bacillus megaterium* or *S. faecalis*. Comparison of the cell and supernatant fractions of the wild-type lactococcal strain and an acmA deletion mutant revealed that all the lytic bands present in the former originated from AcmA, as all had disappeared in the deletion mutant. As the smallest of the active bands corresponded to a molecular size of approximately 29 kDa, a large part of AcmA can be removed without major loss of activity. As the active site resides, most probably, in the N-terminus (see below) the deletions are thought to occur in the C-terminal repeated region. Degradation of cell wall hydrolases, without loss

of activity, has been observed before in *Bacillus licheniformis* (34) and *Bacillus subtilis* (22,23,37). In cell-free extracts of *E. coli* cells expressing *acmA*, two bands, one of 46 and one of 41 kDa, and several smaller bands were present upon renaturing SDS-PAGE using cell walls of *M. lysodeikticus*. Bands of 41 and 46 kDa were also detected in cell-free extract of *L. lactis* but only the smaller of the two was found in the supernatant fraction of *L. lactis*. *acmA* encodes a protein of 437 amino acids and the calculated molecular mass of AcmA (46.564 Da) corresponds to the size of the largest clearing band in the lactococcal cell-free extract (46 kDa). Within the deduced amino acid sequence of AcmA a putative signal sequence of 57 amino acids was identified using the rules of von Heijne (49). In contrast to most signal peptides from Gram-positive bacteria, the n-region of this putative signal sequence consists of 29 amino acids with 12 charged amino acid residues instead of eight to twelve (50). This is similar to the signal sequence of muramidase-2 of *Enterococcus hirae* which has 11 charged amino acids within the first 29 amino acids (6). Based on a signal peptide of 57 amino acids, the molecular mass of mature AcmA would be 40.264 Da, which corresponds to the size (41 kDa) of the major clearing band in the culture supernatant of *L. lactis*. Most probably, AcmA is produced as a preprotein and the secreted form is the 41-kDa protein found in the supernatant of an *L. lactis* culture. The 46-kDa preprotein is either produced in lower amounts or has a reduced activity in the assay used, as the corresponding band of activity is always less clear. The 41-kDa protein that is present in the cell-free extract is, most likely, the enzyme that is still attached (with the C-terminal repeat region (see below)) to the whole cell. The deduced amino acid sequence of AcmA shows overall similarity with muramidase-2 of *Enterococcus hirae* (6) and the autolysin of *S. faecalis* (2). The identity was very high in the N-terminal regions of the three proteins which, most probably, encompass the active site (20). Within this region homology was also found with the flagellar protein FlgJ of *Salmonella typhimurium* (19). In the C-terminal part of AcmA, three repeated regions were present separated by nonhomologous sequences rich in serine, threonine, and asparagine residues. Muramidase-2 possesses six of such repeated regions separated by the same kind of nonhomologous sequences as in AcmA. Within the repeats a consensus sequence was postulated by Joris et al. (20). From the homology comparison it is clear that AcmA, muramidase-2, and the autolysin of *S. faecalis* contain 3, 6, and 5 of such consensus sequence, respectively. All three cell wall hydrolases have a repeat at their extreme C-terminus. Similar repeats have also been detected in the *Bacillus subtilis* .phi.PZA lysozyme, the homologous *Bacillus* gene 15 lysozyme, *Listeria monocytogenes* pathogenicity-associated protein p60, and *Staphylococcus aureus* protein A by Joris et al. (20). and in the sporulation related .tau.-D-glutamyl-(L)meso-diaminopimelic-acid-hydrolysing peptidase I of *Bacillus sphaericus* by Hourdou et al.(16). We also found this consensus twice in the N-terminal part of DniR of *E. coli*, a protein affecting the anaerobic expression of the hexaheme nitrite reductase (21), of which the second repeat showed some similarity with the first repeat of AcmA. The repeated regions are thought to be involved in substrate recognition and, thus, cell-wall binding (20).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KMOC	Draw D
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☐ 10. Document ID: US 5872238 A

L8: Entry 10 of 12

File: USPT

Feb 16, 1999

DOCUMENT-IDENTIFIER: US 5872238 A

TITLE: Thermophile gene transfer

Brief Summary Text (19):

Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431, teach the self-selection of undescribed origins of replication from cryptic plasmids from uncharacterized Thermus spp. and Thermus aquaticus are isolated and cloned into E. coli vectors. Plasmids were constructed with these origins, pLU1 to pLU4 from T. aquaticus, and pMY1 to pMY3 from Thermus spp. The plasmids then had a modified form of the cellulase gene (celA) from Clostridium thermocellum and were expressed in E. coli with the signal peptide from the S-layer gene from T. thermophilus. Transformation back into T. thermophilus allowed for expression at 70.degree. C.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	RMC	Draw. De
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☐ 11. Document ID: US 5786174 A

L8: Entry 11 of 12

File: USPT

Jul 28, 1998

DOCUMENT-IDENTIFIER: US 5786174 A

TITLE: Thermophile gene transfer

Brief Summary Text (20):

Lasa et al. (1992a) Development of Thermus-Escherichia Shuttle Vectors and Their Use for Expression of the Clostridium thermocellum celA Gene in Thermus thermophilus, J. of Bacteriology 174:6424-6431, teach the self-selection of undescribed origins of replication from cryptic plasmids from uncharacterized Thermus spp. and Thermus aquaticus are isolated and cloned into E. coli vectors. Plasmids were constructed with these origins, pLU1 to pLU4 from T. aquaticus, and pMY1 to pMY3 from Thermus spp. The plasmids then had a modified form of the cellulase gene (celA) from Clostridium thermocellum and were expressed in E. coli with the signal peptide from the S-layer gene from T. thermophilus. Transformation back into T. thermophilus allowed for expression at 70.degree. C.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	RMC	Draw. De
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☐ 12. Document ID: US 5004692 A

L8: Entry 12 of 12

File: USPT

Apr 2, 1991

DOCUMENT-IDENTIFIER: US 5004692 A

TITLE: Cloning and expression of phospholipase C genes

Drawing Description Text (2):

FIG. 1 depicts the nucleotide sequence and putative corresponding amino acid sequence of a DNA segment encoding Clostridium perfringens Phospholipase C. The arrow after amino acid 22 indicates a possible processing site of the signal peptide.

Drawing Description Text (3):

FIG. 2 depicts the nucleotide sequence and putative corresponding amino acid sequence of a DNA segment encoding Clostridium bifermentans Phospholipase C. The arrow after amino acid 23 indicates a possible processing site of the signal peptide.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Assignments	Claims	KWIC	Draw D
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Terms	Documents
L7 not L3 not L4	12

**Display Format:** [KWIC](#)[Change Format](#)[Previous Page](#)[Next Page](#)[Go to Doc#](#)



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L11: Entry 15 of 15

File: USPT

Dec 10, 1996

US-PAT-NO: 5583038

DOCUMENT-IDENTIFIER: US 5583038 A

TITLE: Bacterial expression vectors containing DNA encoding secretion signals of lipoproteins

DATE-ISSUED: December 10, 1996

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stover; Charles K.	Silver Spring	MD		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
MedImmune, Inc.	Gaithersburg	MD			02

APPL-NO: 07/ 977630   [PALM]

DATE FILED: November 17, 1992

## PARENT-CASE:

This application is a continuation-in-part of application Ser. No. 780,261, filed Oct. 21, 1991 now abandoned.

INT-CL: [06] C12 N 1/21, A61 K 39/04

US-CL-ISSUED: 435/252.3; 424/93.2

US-CL-CURRENT: 435/252.3; 424/93.2

FIELD-OF-SEARCH: 424/93A, 424/94.4, 424/93.2, 435/252.3, 435/320.1

## PRIOR-ART-DISCLOSED:

## FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO88/06626	September 1988	WO	

## OTHER PUBLICATIONS

Simon et al., J. Infect. Dis., 164:123 (1991).  
Fikrig et al., Science (Wash., D.C.), 250:553 (1990).  
Fikrig et al., Infect. Immun., 60:657 (1992).  
Fikrig et al., Proc. Natl. Acad. Sci. USA, 89:5418 (1992).  
Howe, et al., Infect. and Immun. 54:207-212 (1986).  
Dunn et al., Protein Exp. and Purif., 1:159-168 (1990).  
Schaible et al., Proc. Natl. Aca. Sci., 87:3768-3772 (1990).

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Generate Collection

L11: Entry 15 of 15

File: USPT

Dec 10, 1996

DOCUMENT-IDENTIFIER: US 5583038 A

TITLE: Bacterial expression vectors containing DNA encoding secretion signals of lipoproteins

Brief Summary Text (24):

Antigens which may be encoded include, but are not limited to, Mycobacterium leprae antigens; Mycobacterium tuberculosis antigens; Rickettsia antigens; Chlamydia antigens; Coxiella antigens; malaria sporozoite and merozoite proteins, such as the circumsporozoite protein from Plasmodium berghei sporozoites; diphtheria toxoids; tetanus toxoids; Clostridium antigens; Leishmania antigens; Salmonella antigens; E. coli antigens; Listeria antigens; Borrelia antigens, including the OspA and OspB antigens of Borrelia burgdorferi; Francisella antigens; Yersinia antigens; Mycobacterium africanum antigens; Mycobacterium intracellulare antigens; Mycobacterium avium antigens; Treponema antigens; Schistosoma antigens; Filaria antigens; Pertussis antigens; Staphylococcus antigens; Herpes virus antigens; antigens, and parainfluenza virus antigens; measles virus antigens; Bordetella antigens; Hemophilus antigens; Streptococcus antigens, including the M protein of S. pyogenes and pneumococcus antigens such as Streptococcus pneumoniae antigens; mumps virus antigens; hepatitis virus antigens; Shigella antigens; Neisseria antigens; rabies antigens; polio virus antigens; Rift Valley Fever virus antigens; dengue virus antigens; measles virus antigens; rotavirus antigens; Human Immunodeficiency Virus (HIV) antigens, including the gag, pol, and env proteins; respiratory syncytial virus (RSV) antigens; snake venom antigens; human tumor antigens; and Vibrio cholera antigens. Enzymes which may be encoded include, but are not limited to, steroid enzymes.

## CLAIMS:

1. Recombinant mycobacteria transformed with DNA encoding a polypeptide, said polypeptide comprising a lipoprotein secretion signal sequence and an antigen heterologous to the mycobacteria wherein the lipoprotein secretion signal causes the antigen to be produced as a lipoprotein.

10. Mycobacteria transformed with DNA encoding a polypeptide, said polypeptide comprising a lipoprotein secretion signal sequence and an antigen which elicits antibodies against Borrelia burgdorferi, wherein the lipoprotein secretion signal causes the antigen to be produced as a lipoprotein.

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. Document ID: US 6605431 B1

L3: Entry 1 of 6

File: USPT

Aug 12, 2003

DOCUMENT-IDENTIFIER: US 6605431 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Promoter elements and methods of use

Other Reference Publication (21):

M. C.. Graves et al., "In Vivo and In Vitro Transcription of the Clostridium pasteurianum Ferredoxin Gene: Evidence for "Extended" Promoter Elements in Gram-Positive Organisms," J. Biol. Chem., 261: 11409-11415 (1986).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KMMC	Draw. De
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☐ 2. Document ID: US 5955368 A

L3: Entry 2 of 6

File: USPT

Sep 21, 1999

DOCUMENT-IDENTIFIER: US 5955368 A

TITLE: Expression system for clostridium species

Other Reference Publication (3):

B. Dupuy and A.L. Sonenshein, "Transcriptional Regulation of Clostridium difficile TOXA and TOXB Genes," p. 58, 1997 (Abstract).

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KMMC	Draw. De
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☐ 3. Document ID: US 5759845 A

L3: Entry 3 of 6

File: USPT

Jun 2, 1998

DOCUMENT-IDENTIFIER: US 5759845 A

TITLE: Secretion of clostridium cellulase by E. coli

Brief Summary Text (10):

Preferably, the expression vector of the transformed microorganism provided by the present invention comprises a first DNA sequence coding for a cellulase obtained from a Clostridium strain IY-2 and a second DNA sequence coding for a Clostridium promoter. Thus, the E. coli host is capable of synthesizing and secreting extracellular cellulase at 28.degree. C. The expression of the cellulase at 28.degree. C. suggests that the promoter for that gene or some other promoter-like sequences that can be recognized by E. coli RNA polymerase have also been cloned with the cellulase structural gene. The results further suggests that E. coli RNA polymerase can utilize the cellulase gene's promoter to initiate transcription and subsequent expression of the Clostridium cellulase in E. coli.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw D
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☐ 4. Document ID: US 5496725 A

L3: Entry 4 of 6

File: USPT

Mar 5, 1996

DOCUMENT-IDENTIFIER: US 5496725 A

TITLE: Secretion of Clostridium cellulase by E. coli

Brief Summary Text (12):

Preferably, the expression vector of the transformed microorganism provided by the present invention comprises a first DNA sequence coding for a cellulase obtained from a Clostridium strain IY-2 and a second DNA sequence coding for a Clostridium promoter. Thus, the E. coli host is capable of synthesizing and secreting extracellular cellulase at 28.degree. C. The expression of the cellulase at 28.degree. C. suggests that the promoter for that gene or some other promoter-like sequences that can be recognized by E. coli RNA polymerase have also been cloned with the cellulase structural gene. The results further suggests that E. coli RNA polymerase can utilize the cellulase gene's promoter to initiate transcription and subsequent expression of the clostridium cellulase in E. coli.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw D
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☐ 5. Document ID: US 5418157 A

L3: Entry 5 of 6

File: USPT

May 23, 1995

DOCUMENT-IDENTIFIER: US 5418157 A

TITLE: Recombinant 68,000 dalton collagenase of Clostridium histolyticum

Detailed Description Text (9):

Typically, the DNA segment of the insert is fused contiguously to a transcription-effecting DNA sequence that controls and activates transcription from the Clostridium DNA segment. Generally, such a transcription-effecting DNA sequence is not itself derived from Clostridium and includes a promoter derived from a procaryote or virus infecting a procaryote and capable of activating transcription under regulation in E. coli. Such promoters include, but are not limited to, the E.

coli lac promoter, the E. coli trp promoter, the bacteriophage .lambda. P.sub.L promoter, the bacteriophage T7 promoter, and the tac promoter, a hybrid trp-lac promoter that is regulated by lac repressor (E. Amann, E. J. Brosius & M. Ptashne, "Vectors Bearing a Hybrid trp-lac Promoter Useful for Regulated Expression of Cloned Genes in Escherichia coli," Gene 25, 167 (1983)). The use of an external transcription-affecting sequence is not required in all cases, particularly when only the 68 kd form of collagenase is produced in larger quantities. This is because because that form of collagenase can be transcribed as a result of the action of an internal promoter lying within the first part of the Clostridium collagenase DNA insert itself. This promoter can control the transcription of that portion of the insert downstream from it to yield mRNA capable of being translated to yield the 68 kd form of collagenase, without the functioning of any promoter external to the DNA derived from C. histolyticum. Because the Clostridium promoter is actually located within the structural gene for the large form of collagenase, it cannot serve to activate transcription of that form.

Detailed Description Text (29):

In this arrangement, the 68 kd form of collagenase is synthesized as a result of transcription activated by the Clostridium promoter that is located within the inserted DNA; the larger fusion peptide is under the control of the lac promoter, to which the segment of the .beta.-galactosidase gene is operatively linked. The 68 kd form of collagenase is not linked to any protein derived from E. coli.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachment	Claims	KMC	Draw. De
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☐ 6. Document ID: US 5177017 A

L3: Entry 6 of 6

File: USPT

Jan 5, 1993

DOCUMENT-IDENTIFIER: US 5177017 A

TITLE: Molecular cloning of the genes responsible for collagenase production from Clostridium histolyticum

Detailed Description Text (9):

Typically, the DNA segment of the insert is fused contiguously to a transcription-affecting DNA sequence that controls and activates transcription from the Clostridium DNA segment. Generally, such a transcription-affecting DNA sequence is not itself derived from Clostridium and includes a promoter derived from a procaryote or virus infecting a procaryote and capable of activating transcription under regulation in E. coli. Such promoters include, but are not limited to, the E. coli lac promoter, the E. coli trp promoter, the bacteriophage .lambda. P.sub.L promoter, the bacteriophage T7 promoter, and the tac promoter, a hybrid trp-lac promoter that is regulated by lac repressor (E. Amann, E. J. Brosius & M. Ptashne, "Vectors Bearing a Hybrid trp-lac Promoter Useful for Regulated Expression of Cloned Genes in Escherichia coli," Gene 25, 167 (1983)). The use of an external transcription-affecting sequence is not required in all cases, particularly when only the 68 kd form of collagenase is produced in larger quantities. This is because that form of collagenase can be transcribed as a result of the action of an internal promoter lying within the first part of the Clostridium collagenase DNA insert itself. This promoter can control the transcription of that portion of the insert downstream from it to yield mRNA capable of being translated to yield the 68 kd form of collagenase, without the functioning of any promoter external to the DNA derived from C. histolyticum. Because the Clostridium promoter is actually located within the structural gene for the large form of collagenase, it cannot serve to activate transcription of that form.

Detailed Description Text (29):

In this arrangement, the 68 kd form of collagenase is synthesized as a result of transcription activated by the Clostridium promoter that is located within the inserted DNA; the larger fusion peptide is under the control of the lac promoter, to which the segment of the .beta.-galactosidase gene is operatively linked. The 68 kd form of collagenase is not linked to any protein derived from E. coli.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Generate Doc	Attachments	Claims	KWIC	Draw D
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